PROCEEDINGS
OF
The American Microscopical Society.

PHOTOMICROGRAPHS BY GAS-LIGHT.

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Photomicrographs are superior to hand-made drawings when they show in a satisfactory manner the structural details of microscopic objects, because they exclude those errors which result from faulty drawing, careless observation, or suppression and exaggeration of details due to personal bias. They are unimpeachable evidence of what has been seen under the microscope, and as such will always have a special value as illustrations for original research work relating to the morphology of microörganisms or histological details of animal and vegetable tissues.

The art of making photomicrographs had its origin in this country, at the Army Medical Museum, in Washington, where Curtis made the first successful efforts and Woodward achieved remarkable success in photographing difficult test diatoms, etc.

Dr. Robert Koch, the famous German bacteriologist, first employed this method in the illustration of his papers relating to bacteria. and published many admirable photomicrographs as long ago as 1877. The present writer, as a member of the Havana Yellow Fever Commission of the National Board of Health, went to Cuba in 1879 provided with a complete outfit for making photomicrographs, including the one-twelfth and the one-eighteenth inch homogeneous oil immersion objectives of Zeiss.

[Extracts from report of Havana Commission, 1879.]

"In Havana Dr. Sternberg gave a large share of his time to the microscopic examination and photography of the blood. No chemical examination was attempted. The patients from whom specimens of blood were obtained were mostly soldiers in the military hospital of San Ambrosio. Ninety-eight specimens from 41 undoubted cases of yellow fever were carefully studied and 105 photographic negatives were made, which show satisfactorily everything demonstrable by the microscope.

"These photographs were mostly made with a magnifying power of 1,450 diameters, obtained by the use of Zeiss' one-eighteenth inch objective and Tolles' amplifier. Probably no better lens than the Zeiss one-eighteenth (oil immersion) could have been obtained for this work, and it is doubtful whether any objective has ever been made capable of showing more than is revealed by this magnificent lens. With the power used, organisms much smaller than those described as existing in the blood of charbon or of relapsing fever would be clearly defined.

"If there is any organism in the blood of yellow fever demonstrable by the highest powers of the microscope as at present perfected the photomicrographs taken in Havana should show it. No such organism is shown in any preparation photographed immediately after collection; but in certain specimens, kept under observation in culture cells, hyphomycetous fungi and spherical bacteria made their appearance after an interval of from 1 to 7 days. The appearance of these organisms was, however, exceptional, and in several specimens taken from the same individual at the same time it occurred that in one or two a certain fungus made its appearance and in others it did not. This fact shows that the method employed cannot be depended upon for the exclusion of atmospheric germs, but does not affect the value of the result in the considerable number of instances in which no development of organisms occurred in culture cells in which blood in a moist state was kept under daily observation for a week or more."

In Cuba I used sunlight, reflected by a heliostat, and, following the method of Woodward, worked in a dark room, which constituted the camera, the enlarged image being projected upon a screen, which was set up in the room at a distance from the microscope. The light was passed through a cell containing a solution of ammonia-sulphate of copper. The results obtained were entirely satisfactory, and I should not be disposed to use any other light than sunlight if this was always available; but we cannot command this light at all times and places, and it often happens that when we are ready to devote a day to making photomicrographs the sun is obscured by clouds or the atmosphere is hazy. Indeed, in some latitudes and at certain seasons of the year a suitable day for the purpose is extremely rare. The use of sunlight, reflected by a heliostat, also requires a

room having a southern exposure and elevated above all surrounding buildings or other objects by which the direct rays of the sun would be intercepted. Again, the only time available for work may be after sunset. For these reasons a satisfactory artificial light is very desirable.

Woodward showed as long ago as 1870 that satisfactory results may be obtained by the use of the oxyhydrogen lime light, the magnesium light, or the electric arc light. I have myself made extensive use of the oxyhydrogen lime light, and consider this the best substitute for sunlight with which I am familiar. The only objections to its use are the considerable expense attending it and the inconvenience resulting from the necessity of having the supply of gas renewed at frequent intervals when much work is being done.

The electric light would probably be less expensive and more convenient if one had at hand an electric-lighting plant, but otherwise the expense attending its use would be too great for most of those who would desire to use it; and if the wires of an electric-lighting plant were within easy reach the light might not be available during the day-time on account of the dynamos not being in motion.

These considerations have led some microscopists to use an oil-lamp as a source of illumination, and admirable results, with comparatively high powers, have been obtained by Governor Cox, Dr. Mercer, Mr. Walmsley, and others.

For photographing diatoms and other objects which do not require the use of a color screen a good oil-lamp is a satisfactory source of illumination; but to photograph bacteria, which are usually stained with fuchsin, gentian violet, or methylene blue, it is necessary to use a yellow or green color screen, and this increases the time of exposure from four to six fold, inasmuch as the actinic rays from the violet end of the spectrum are stopped out by the color screen. Under these circumstances the use of an oil-lamp, in my experience, has not proved to be practicable, on account of the feebleness of the illumination and the long exposures required.

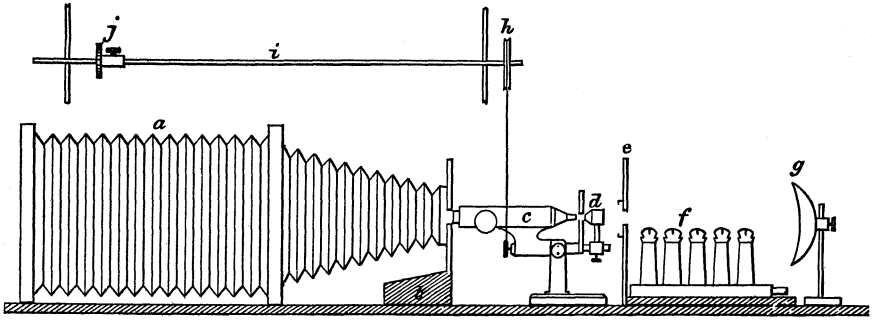
These considerations led me—in 1889, while preparing my report of the investigations which I had recently made in Cuba—to experiment with gas-light, and, as I obtained very satisfactory results with the apparatus devised for the purpose, I have thought it worth while to call the attention of the members of the American Microscopical Society to the advantages of the method.

My photomicrographs have been made with the three-millimeter oil-immersion apochromatic objective of Zeiss and his projection

eye-piece No. 3. I make all of my photomicrographs of bacteria with a standard amplification of 1,000 diameters. Most of them have been made from preparations stained with a simple aqueous solution of fuchsin (freshly prepared) or with cahol-fuchsin solution ("Ziehl's solution").

I am in the habit of using a yellow screen, placed back of the achromatic condenser (Abbe's), which is prepared by coating a plate of glass with a film of negative varnish in which tropæolin has been dissolved. Success depends largely upon the use of good orthochromatic plates. My photomicrographs have been made with orthochromatic plates manufactured by Carbutt, of Philadelphia.

I use a large Powell and Lealand stand, upon the substage of which I have fitted an Abbe condenser. The arrangement of the apparatus will be understood by reference to the accompanying figure:



"A is the camera, which has a pyramidal bellows front supported by the heavy block of wood B. This can be pushed back upon the baseboard which supports it, so as to allow the operator to place his eye at the eye-piece of the microscope. When it is brought forward an aperture of the proper size admits the outer extremity of the eye-piece and shuts off all light except that coming through the objective. C is the microscope, and D the Abbe condenser supported upon the substage; E is a thick asbestos screen for protecting the microscope from the heat given off by the battery of gas-burners F. This asbestos screen has an aperture of proper dimensions to admit the light to the condenser D. The gas-burners are arranged in a series, with the flat portion of the flame facing the aperture in the asbestos screen E. The concave metallic mirror G is properly placed to reflect the light in the desired direction. I have not found

any advantage in the use of a condensing lens other than the Abbe condenser upon the substage of the microscope. The focusing is accomplished by means of the rod I, which carries at one extremity a grooved wheel, H, which is connected with the fine-adjustment screw of the microscope by means of a cord.

"The focusing wheel J may be slipped along the rod I to any desired position, and is retained in place by a set-screw. The rod I is supported above the camera by arms depending from the ceiling or by upright arms attached to the baseboard.

"I have lost many plates from a derangement of the focal adjustment resulting from vibrations caused by the passing of loaded wagons in the street adjoining the laboratory in which I work. This has been overcome to a great degree by placing soft rubber cushions under the whole apparatus." *

For the convenience of those who may wish to make themselves familiar with what has been done in this country and in Europe in the way of perfecting the art of photomicrography I append the following bibliography taken from my recently published "Manual of Bacteriology" (Wm. Wood & Co., New York):

156. WOODWARD, J. J. Report to the Sugeon-General of the United States Army on the magnesium and electric lights as applied to photomicrography. Washington, 1870.

157. KOCH. Verfahren zur Untersuchung, zum Conserviren, und Photographiren der Bakterien. Cohn's Beiträge zur Biol. der Pflanz., Bd. ii, Heft 3, 1877.

158. STERNBERG. Photomicrographs, and how to make them. Boston, 1884, 204 pages, 20 plates.

159. ———. Photomicrography by gas-light. Johns Hopkins University Circulars, vol. ix, No. 81, p. 72.

160. COX, J. D. On some photographs of broken diatom valves, taken by lamp-light. Journ. R. M. S., London, 1884, p. 853.

161. CROOKSHANK. Photography of bacteria. Illustrated by 86 photographs reproduced by autotype. London, 1887.

162. ISRAEL. Ueber Mikrophotographie mit starken Objectivsystemen. Virchow's Arch., Bd. cvi, p. 502.

163. NEUHAUSS. Anleitung zur Mikrophotographie für Arzte, Botaniker, etc. Berlin, 1887.

164. ———. Die Entwickelung der Mikrophotographie, etc. Centralbl. für Bakteriol., Bd. iv, pp. 81 and 111.

165. ———. Verschiedenes über Mikrophotographie. Zeitschr. für wiss. Mikroskopie und für mikrosk. Technik, Bd. v, p. 484.

* From Johns Hopkins University Circulars, vol. ix, No. 81, p. 72.

166. GÜNTHER. Photogramme der pathogenen Mikroorganismen. Berlin, 1887.
167. KITT. Ueber Mikrophotographien. Oesterr. Monatschr. für Thierheilk., 1888, p. 241.
168. FRÄNKEL UND PFEIFFER. Mikrophotographischer Atlas der Bakterienkunde. Berlin, 1889.
169. COMBER. On a simple form of heliostat, and its appliances in photomicrography. Journ Roy. Mic. Soc., London, August, 1890, p. 429.
170. MARKTANNER UND TURNERETSCHER. Die Mikrophotographie. Halle, 1890, 344 pp., 195 engravings.

Description of Plates.

FIG. 1.—Micrococcus ("staphylococcus"), supposed by Dr. Domingos Freire, of Brazil, to be the specific cause of yellow fever and given to Dr. Sternberg at the time of his visit to Rio de Janeiro (1887) by Dr. Freire. Proved by the investigations of Dr. Sternberg not to be concerned in the etiology of yellow fever. Stained with fuchsin. × 1,000 diameters.

FIG. 2.—Micrococcus in tetrads (*Mic. tetragenus versatilis*, Sternberg), supposed by Dr. Finlay, of Havana, to be the specific cause of yellow fever. Proved by the investigations of Dr. Sternberg not be concerned in the etiology of this disease (see report on the etiology and prevention of yellow fever, Washington, 1890). Stained with fuchsin. × 1,000 diameters.

FIG. 3.—*Bacillus cuniculicida havanientis* (Sternberg). Stained with fuchsin. × 1,000 diameters.

FIG. 4.—*Bacillus acidiformans* (Sternberg). Stained with fuchsin. × 1,000 diameters.

FIG. 5.—*Bacillus E.* (Sternberg). "A motionless, liquefying bacillus, obtained from the fæces of a healthy individual" (Havana, 1889). Stained with fuchsin. × 1,000 diameters.

FIG. 6.—*Bacillus vacuolosis* (Sternberg). Stained with fuchsin. × 1,000 diameters. For a detailed account of the bacteria above referred to, see Dr. Sternberg's "Manual of Bacteriology." (Wm. Wood & Co., New York, 1892.)

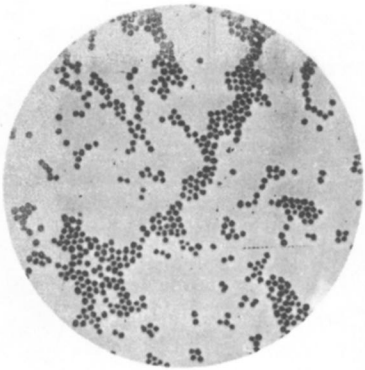


FIG. 1.

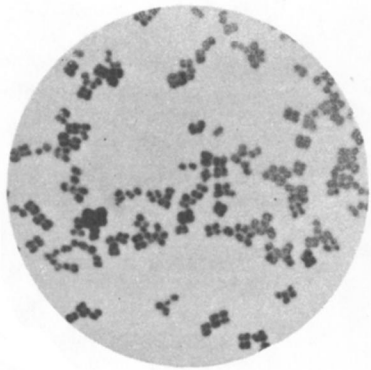


FIG. 2.

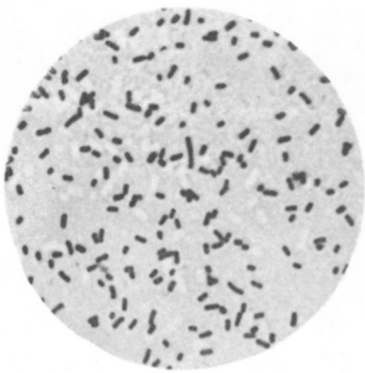


FIG. 3.

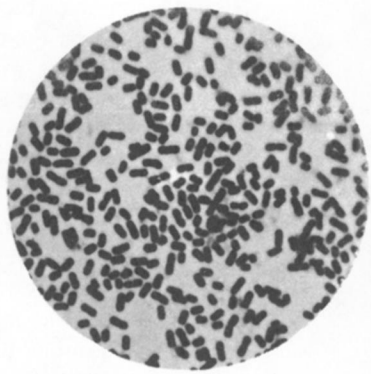


FIG. 4.

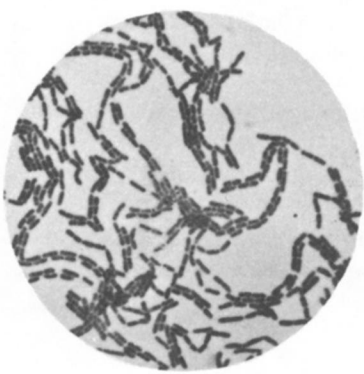


FIG. 5

FIG. 6.